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Reactivation of the Same Synapses during Spontaneous Up States and Sensory Stimuli

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SUMMARY

In the mammalian brain, calcium signals in dendritic spines are involved in many neuronal functions, particularly in the induction of synaptic plasticity. Recent studies have identified sensory stimulation-evoked spine calcium signals in cortical neurons in vivo. However, spine signaling during ongoing cortical activity in the absence of sensory input, which is essential for important functions like memory consolidation, is not well understood. Here, by using in vivo two-photon imaging of auditory cortical neurons, we demonstrate that subthreshold, NMDA-receptor-dependent spine calcium signals are abundant during up states, but almost absent during down states. In each neuron, about 500 nonclustered spines, which are widely dispersed throughout the dendritic field, are on average active during an up state. The same subset of spines is reliably active during both sensory stimulation and up states. Thus, spontaneously recurring up states evoke in these spines "patterned" calcium activity that may control consolidation of synaptic strength following epochs of sensory stimulation.

INTRODUCTION

Recent work has provided insights into the sensory activation of cortical synapses in vivo by characterizing local dendritic hot spots in the visual cortex (Jia et al., 2010) and single spines in the auditory and vibrissal cortex (Chen et al., 2011; Varga et al., 2011) (for review, see Grienberger and Konnerth, 2012; Rochefort and Konnerth, 2012). By using two-photon microscopy (Denk et al., 1990) in combination with whole-cell recordings (Jia et al., 2010, 2011), subthreshold calcium signals were detected in restricted dendritic segments ("hot spots") in layer 2/3 (L2/3) neurons of mouse visual cortex in response to visual stimulation. These hot spots, representing synaptic entry sites originating mainly in other cortical neurons, were mostly well tuned to the direction and orientation of the visual stimulus. Important insights from this study include the observations that each cortical neuron, without exception, receives inputs tuned for multiple orientations and directions (Jia et al., 2010). Two more recent studies used two-photon microscopy involving the sensitive method of low-power temporal oversampling (LOTOS) (Chen et al., 2011, 2012) to characterize sensory-evoked calcium signals in individual spines of neurons in both mouse auditory (Chen et al., 2011) and vibrissal cortex (Varga et al., 2011). As shown for the case of visual cortical dendritic hot spots, spine calcium signals that were activated by sensory stimulation showed a heterogeneous distribution with even neighboring spines coding mostly for different sensory features (Chen et al., 2011; Varga et al., 2011). Importantly, subthreshold spine calcium signals required N-methyl-D-aspartate receptor (NMDAR) activation. In many, but not all, spines, NMDAR-activation-dependent calcium signals represented a characteristic feature that could be evoked repeatedly in consecutive trials (Chen et al., 2011; Varga et al., 2011). In view of the essential role of spine calcium signals for synaptic plasticity established in vitro (reviewed in Sabatini et al., 2001; Yuste et al., 2000; Zucker, 1999), such NMDAR-dependent calcium entry signals may be important for the induction of plastic modification of synaptic transmission in those spines that are effectively activated by sensory stimuli in vivo.

In contrast to the accumulating knowledge of sensory-dependent activation of single spines, little is known about subthreshold spine calcium signaling in cortical neurons during spontaneous activity in vivo. It is well known that, throughout the mammalian brain, ongoing neuronal activity is spontaneously present even without intentional sensory activation (Kenet et al., 2003; Lestienne, 2001; Ringach, 2009; Steriade et al., 2001). In early development, different forms of spontaneous activity are considered to be important for the refinement of immature circuits in various brain regions (Garaschuk et al., 2000; Katz and Shatz, 1996; Thompson, 1997; Weliky and Katz, 1999). Later in development, spontaneous activity has been best studied in the cortex in conditions of slow-wave sleep, anesthesia, or quiet wakefulness, where it consists of alternating up and downstates, termed slow oscillation (Cowan and Wilson, 1994; Petersen et al., 2003; Steriade et al., 1993, 2001). This patterned cortical activity is thought to be essential for sensory signal processing by providing an internal representation of previously stored

information (Arieli et al., 1996; Kenet et al., 2003; Kisley and Gerstein, 1999). In addition, slow oscillations have been suggested to be directly involved in memory consolidation by giving a context for the replay of previous experience (Ji and Wilson, 2007; Marshall et al., 2006). Memory consolidation may require repeated NMDAR activation (Aton et al., 2009; Gais et al., 2008; Shimizu et al., 2000) during ongoing activity, leading to calcium signaling and long-term-potentiation (LTP)-like modifications in the relevant synapses (McGaugh, 2000).

To characterize calcium signals in spines during spontaneous activity in vivo, we used LOTOS-based two-photon calcium imaging in combination with electrophysiological recordings in layer 2 (L2) pyramidal neurons in the auditory cortex of anesthetized mice. We show that a subset of spines exhibits NMDAR-dependent calcium signals during subthreshold up states but show almost no calcium signaling during down states. The active spines are spatially distributed throughout the dendrites and temporally dispersed during individual up-state events. We estimate that more than 500 active spines are required for generating an up state in an L2 neuron. Importantly, the same sets of spines exhibit both sensory-evoked and spontaneous calcium responses with a similar response rate. Thus, our results identify recurrently occurring NMDAR-activation-dependent calcium signals that occur reliably in a subset of spines in cortical neurons in vivo during slow oscillations. We suggest that these calcium signals represent a cellular substrate for synaptic plasticity associated with memory consolidation.

RESULTS AND DISCUSSION

In Vivo Recording of Subthreshold Calcium Signals in Dendritic Spines during Up States

To analyze spine signaling during spontaneous activity in vivo, we performed two-photon imaging in combination with whole-cell recordings in L2 pyramidal neurons in the primary auditory cortex of isoflurane-anesthetized mice (Chen et al., 2011, 2012; Jia et al., 2010). Somatic current-clamp recordings revealed spontaneous fluctuations of the membrane potential (Vm) between depolarized states (up states) and hyperpolarized states (down states) (Figure 1A, left), thereby generating a bimodal distribution of membrane potentials (Figure 1A, right). The spontaneous up states occurred at frequencies ranging from 0.23 to 0.83 Hz (0.45 ± 0.03 Hz; n = 10 cells) (Figure 1B), in line with the slow oscillations that occur during natural slow-wave sleep, anesthesia, or quiet wakefulness (Petersen et al., 2003; Steriade et al., 1993). By using the LOTOS variant of two-photon Ca^{2+} imaging in vivo (Chen et al., 2011, 2012), we monitored Ca^{2+} signals in single dendritic spines of L2 neurons (Figure 1C). In the absence of action potentials (APs), Ca^{2+} transients were observed during spontaneous up states at different frequencies in different spines (S1 through S4 in this experiment, Figure 1D). The failure of such subthreshold Ca^{2+} transients in spine S3 was not due to functional impairments, because APs reliably produced calcium transients in all spines, including S3 (Figure 1D, right column).

The response rate of a spine, defined here as the ratio of the number of up states with (one or more) subthreshold Ca^{2+} transients and the total number of up states, varied widely between different spines (Figures 1C–1E). For example, in the experiment depicted in Figure 1D, spine S1 had a response rate of 1, whereas spine S4 exhibited only a single large Ca^{2+} transient, whereas spine S3 was silent (response rate = 0). For further analysis, we assigned the spines to three groups: (1) spines with "high" response rates ($\geq$0.5 Ca^{2+} transient/up state), (2) spines with "low" response rates (<0.5 Ca^{2+} transient/up state), and (3) silent spines (no spontaneous Ca^{2+} transients). With this classification, 29% of the imaged spines were found to have a high and 61% a low response rate, whereas 10% were silent (n = 207 spines from ten neurons). Spine calcium signals reflect spike activity in the connected presynaptic neurons. Thus, the larger proportion of spines with a low response rate might receive inputs from neurons in the same layer (Binzegger et al., 2004; Feldmeyer et al., 2006; Petreanu et al., 2009), which are known to have sparse spike activity in vivo (de Kock et al., 2007; Sakata and Harris, 2009).

The results indicate that about 10% of the imaged spines are silent in terms of calcium signals during cortical up states. One concern would be whether these spines are truly silent or the sensitivity of our imaging technique is not sufficient to detect very small calcium signals. Previous recordings from slice preparations have shown that calcium responses in dendritic spines evoked by single excitatory postsynaptic potentials (EPSPs) are about twice as large as those evoked by backpropagation of a single-action potential (Holthoff et al., 2004; Kovalchuk et al., 2000; Nevian and Sakmann, 2004; Yuste and Denk, 1995). Therefore, we considered the detection of single-action potential backpropagation-evoked spine calcium signals as the criterion for judging the sensitivity of our imaging system to detect synaptic calcium responses. To try to avoid contamination from synaptic signals, we analyzed only the single-action potential occurring at the end of up state (Figure S1). Overall, we recorded ten such single-action potentials and detected calcium signals in the silent spines during all these ten events (calcium amplitude = 0.26 ± 0.02 Δf/f). Thus, it is unlikely that with our imaging procedure we have considerably missed synaptic calcium signals.

To explore the spatial distribution of active spines during up states, we imaged dendritic segments throughout most of the dendritic field of L2 neurons, ranging from apical dendrites, close to the brain surface, down to basal dendrites, up to a depth of 300 μm. Figure 1F shows an example in which we obtained recordings from nine dendritic segments in the same neuron. These recordings together with recordings in nine other neurons showed that spine calcium signals during cortical up states with different response rates are widely distributed throughout the dendritic field. The data suggest that spatially distributed synaptic inputs may be required for the membrane depolarization observed during up states. Thus, dendritic spine activation that contributes to the generation of up states in this type of neurons is a feature of both apical and basal dendrites.

Subthreshold Calcium Signals in Dendritic Spines during Up States Are Synaptically Driven

Based on in vitro work in slice preparations, synaptically driven calcium signals in dendritic spines of cortical pyramidal neurons are generated by several sources, including activation of NMDA glutamate receptors, non-NMDA glutamate receptors,

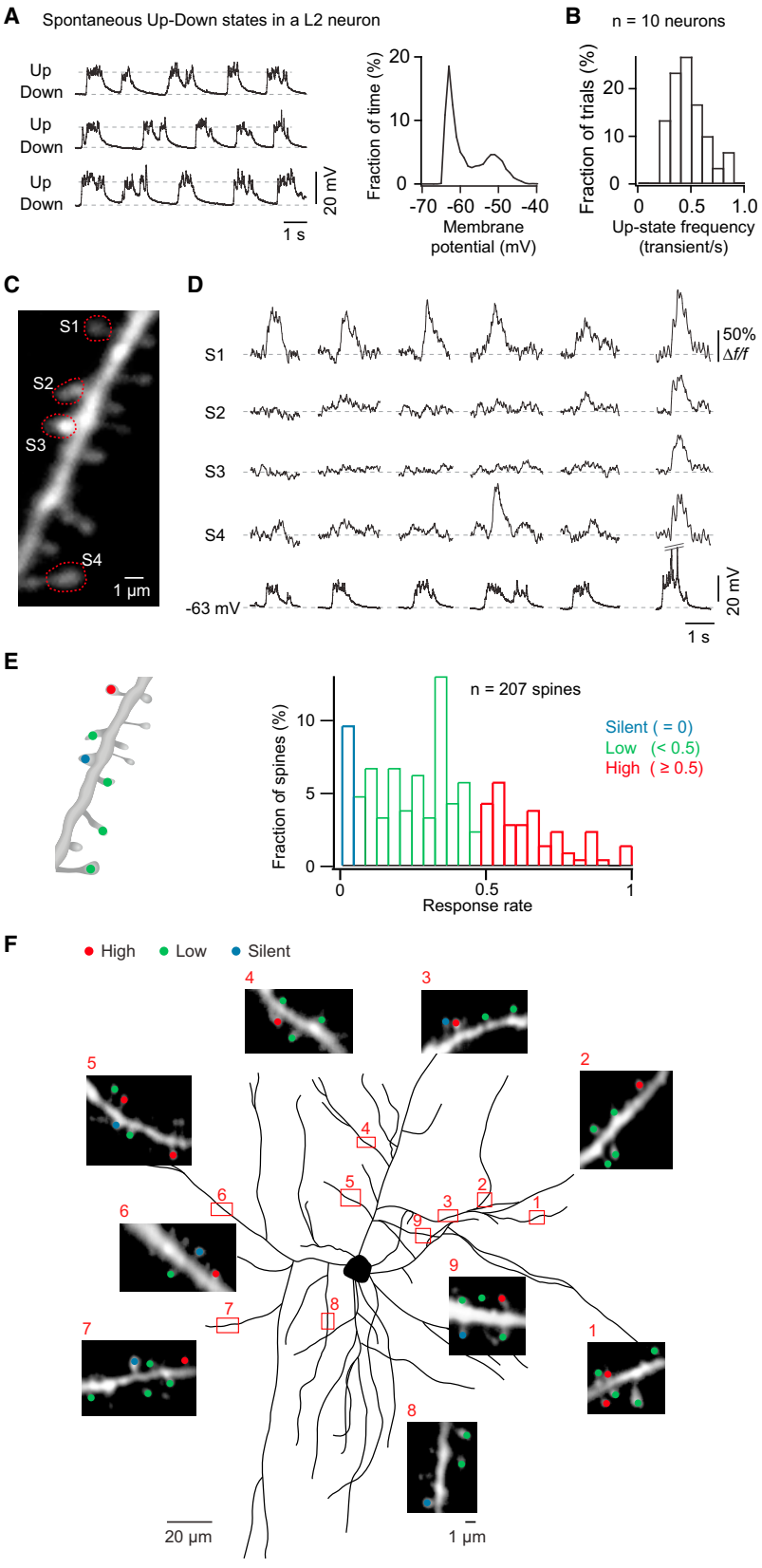

Figure 1. Single Spine Calcium Signals during Spontaneous Up States

(A) Spontaneous up and down states recorded in the soma of a L2 cortical pyramidal neuron in the primary auditory cortex. Left: examples of electrical traces; right: distribution of membrane potentials of this neuron during a 32 s recording period.

(B) Distribution of up-state frequency, calculated from three trials for each neuron (n = 10 neurons).

(C) Two-photon image of a dendritic segment (average of 6,250 frames, frame rate = 1,000 frames/s^{-1}).

(D) Subthreshold calcium transients in the dendritic spines (S1–S4) indicated in (C) during five up states. Last column: action potential-evoked calcium transients. Note that for displaying subthreshold up states on a large scale, spikes in the last column are truncated in amplitude. The neuron was held at resting membrane potential.

(E) Classification of spines based on their response rates. Left: cartoon showing three classes of spines: spines with "high" response rates marked in red, spines with "low" response rates marked in green, and silent spines marked in blue. This is the same dendrite as shown in (C) and (D). Right: distribution of the response rate of spine calcium signals. Data were from 207 imaged spines.

(F) Spatial distribution of active spines during up states in the dendritic field of a L2 cortical pyramidal neuron. Reconstruction of a neuron with dendritic recording sites marked and numbered, surrounded by corresponding insets indicating spine activity. Insets show the two-photon image of the corresponding dendritic segments, indicating with colored dots the spines that had high response rates (red), low response rates (green), and no response (blue). See also Figure S1.

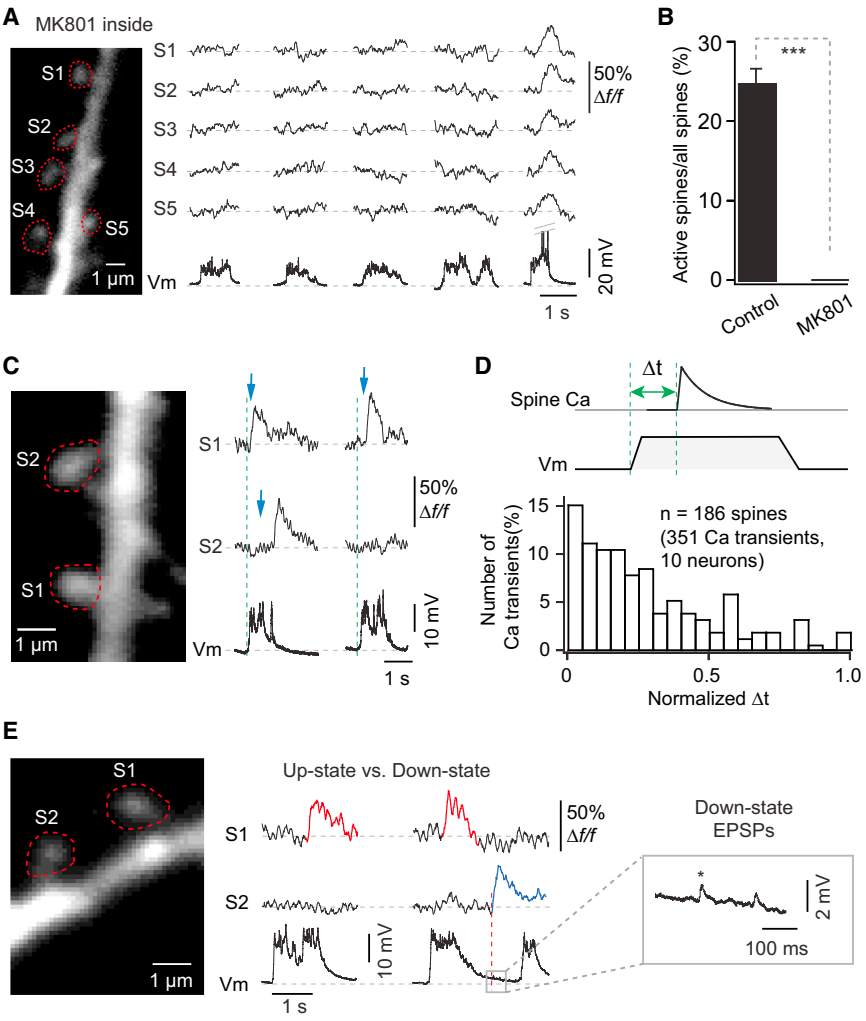

Figure 2. Synaptic Origin and Temporal Distribution of Spine Calcium Signals during Slow Oscillations

(A) Left: two-photon image of a dendritic segment (average of 6,250 frames). Right: calcium signals recorded in the spines (S1–S5) indicated in the left panel, during spontaneous up states and action potentials in the presence of intracellular MK-801 (1 mM). Note that for clarity of the subthreshold up states, spikes in last column are truncated.

(B) Percentage of active spines during up states in control condition (n = 30 dendritic segments from ten neurons) and in the presence of intracellular MK-801 (n = 12 dendritic segments from four neurons). Unpaired t test, ***$p < 0.0001$. Error bars show SEM.

(C) Example of spine calcium signals during up states. Note the variable latencies (pointed out by blue arrows) of the onset of calcium transients with respect to the onset of the up states.

(D) Distribution of the latency of the onset of calcium transients during up states. Δt is the time from the onset of the up state to the onset of the spine calcium transient. Δt was normalized to the duration of each corresponding up state.

(E) Spine calcium signals during up states (S1, marked in red) and during a down state (S2, marked in blue). Inset: expanded scale of the somatic EPSP marked with an asterisk, temporally corresponding to the calcium response in spine S2.

See also Figure S2.

voltage-gated calcium channels, and calcium release from internal stores (Grienberger and Konnerth, 2012; Higley and Sabatini, 2012). Among these, NMDAR-dependent calcium entry is the major source of synaptic calcium signals in spines in different cell types (Higley and Sabatini, 2012; Kovalchuk et al., 2000; Schiller et al., 1998). To confirm whether the subthreshold calcium signals in spines observed during up states are of synaptic origin, we applied intracellularly MK-801 (1 mM), a NMDAR channel antagonist. We found that MK-801 completely blocked spontaneous subthreshold calcium transients in spines, but did not abolish calcium transients evoked by backpropagating action potentials (Figures 2A and 2B). This NMDAR dependence of calcium transients observed during spontaneous subthreshold activity is also true for spine calcium signaling evoked by sensory stimulation in this type of neuron (Chen et al., 2011). It is important to note that NMDAR-dependent calcium signals in spines may be generated by calcium influx through NMDAR channels themselves, but there can be also a contribution from NMDAR-dependent depolarization-mediated activation of voltage-gated calcium channels (Bloodgood et al., 2009; Higley and Sabatini, 2012). However, unlike the results in striatal spiny projection neurons from in vitro experiments (Kerr and Plenz, 2002), calcium signals that were widely distributed in dendrites during the transitions from down states to up states, indicative of calcium entry through voltage-gated calcium channels, were rarely encountered (Figures 1D, 2C, and 2E). In contrast to the major contribution of NMDAR activation to the spine calcium signals, we could still observe prominent bimodal membrane potential fluctuations in the presence of MK-801 (Figures S2A and S2B), suggesting that there is only a minor contribution of NMDAR-mediated conductance to the spontaneous up and down states in this type of neurons, whereas the AMPA-receptor-mediated conductance is the probably major contributor for the depolarization observed during up states.

Temporal Distribution of Subthreshold Spine Calcium Signals during Up and Down States

Previous electrical recordings of somatic synaptic events indicate that up states may result from barrages of afferent synaptic activity (Shu et al., 2003; Steriade et al., 1993). This view then suggests that synaptic inputs onto dendritic spines may occur throughout the entire duration of an up-state event. Alternatively, synaptic activity may initiate the up state, whereas voltage-dependent mechanisms may account for the late phase of the

up-state events (Plotkin et al., 2011). The latency analysis of calcium transients during up states revealed a wide range of delays (Figure 2C, marked by blue arrows), and even in the same spines the delays were variable with respect to the onset of the up state during different trials (e.g., spine S1 in Figure 2C). A quantitative analysis yielded, unexpectedly, a monotonically decreasing distribution of latencies, with a larger number of spines activated at the early stage of up states, followed by a progressive decrease of the number of active spines (Figure 2D, n = 186 active spines from ten neurons). Thus, the data provide direct proof that active synaptic inputs, at least the NMDAR-dependent ones, are temporally distributed throughout the entire up state. These results indicate also that the continuous synaptic barrages are not uniform across single up-state events, with more synaptic inputs occurring at the beginning of up state.

The mechanism underlying down states is not entirely clear. Down states, interrupted by up states, may be either mediated by increased synaptic inhibition (Shu et al., 2003; Steriade et al., 1993) or may represent periods lacking excitatory synaptic activity. Our in vivo imaging approach of excitatory input calcium signals at dendrites would be a clear way to differentiate between these two possibilities. Earlier reports involving somatic and dendritic electrical recordings have shown that EPSPs are present during both up states and down states (Steriade et al., 1993; Waters and Helmchen, 2004). In our recordings from neuronal somata, we also detected small EPSPs during down states (Figure 2E, inset), ranging from 0.43 to 2.32 mV in amplitude (0.99 ± 0.04 mV; n = 10 neurons; see Figure S2C). At simultaneously imaged dendritic sites, we, occasionally, observed calcium responses in single spines temporally corresponding to the small EPSPs (Figure 2E). Overall, we found that only 5% of the imaged spines exhibited calcium responses during down states throughout all recordings (n = 10/194 spines from ten neurons; eight spines were active only during down states, and two spines were active during both up and down states). Because NMDAR activation is voltage dependent, one could argue that the weak synaptic activity detected in these experiments only reflects a reduced number of open NMDAR channels in active synapses during down states. This is, however, unlikely because previous studies indicated that NMDAR-dependent local dendrite (Jia et al., 2010) and spine calcium signals (Chen et al., 2011; Varga et al., 2011) can be reliably detected if the neurons are hyperpolarized to −75 mV or below, which suggests that AMPA-receptor-dependent depolarization in spines is sufficiently large for the activation of NMDA receptor channels even at hyperpolarized membrane potentials. Therefore, this relatively small number of spine calcium signals observed during down states is mostly due to a reduced excitatory synaptic drive during down states (as indicated by voltage-clamp recording in Figure S2D, see also Waters and Helmchen, 2004). The sparse synaptic activity associated with down states may reflect activity originating perhaps in connected subcortical neurons.

Number of NMDAR-Dependent Synaptic Inputs Required for Single Up States

Up states represent "windows of opportunity" for corticothalamic integration, allowing neurons to receive and transmit information with long-range distances (Compte et al., 2008; Wang et al., 2010). Therefore, it is of interest to find out how many NMDAR-dependent synapses on a cell, representing a correlate of the connected presynaptic neurons, are on average active during an up state. To address this issue, we identified in each imaged dendritic segment those spines that were at least active once during an individual up state. Figures 3A and 3B illustrates the results obtained in two dendritic segments, in which one to four spines were found to be active during a single up state. Overall, during an average up-state event (duration: 0.74 ± 0.02 s; n = 150 up-state events from ten neurons), we obtained a median number of 11 active spines per 100 μm of dendritic length (n = 30 dendrites from ten neurons; five consecutive up states for each dendrite) (Figures 3C–3E). This analysis includes both classes of spines with "high" and "low" response rates. We next determined from reconstructed biocytin-labeled neurons the total dendritic length and found it to be about 4.4 mm (4.4 ± 0.3 mm; n = 3 neurons) (Figure 3F). Therefore, when extrapolating the results obtained in the individual dendritic segments and considering that we may underestimate the number of active spines, we conclude that in a L2 mouse auditory cortical neuron more than 500 spines are active during single up-state events. This number corresponds to less than one active spine per millisecond of up states, which is about three times more than what has been estimated from the analysis of a compartmental model of L2/3 pyramidal neurons (Waters and Helmchen, 2006). Although the spine density may be variable in different dendritic regions, the number of active spines seems to be largely uniformly distributed at dendritic sites located no more than 300 μm away from the soma (Figure S3).

Sensory-Evoked and Spontaneous Activity in the Same Spines

There is increasing evidence suggesting that slow cortical oscillations contribute to experience-dependent plasticity in sensory cortical regions (Diekelmann and Born, 2010; Gais et al., 2008; Ji and Wilson, 2007; Stickgold, 2005). A prerequisite for such a mechanism is that the same synapses are active during both sensory stimulation and spontaneous up states. To test this hypothesis, we imaged the same spiny dendritic segment during sound stimulation and also during spontaneous activity. We found, in line with what had been previously reported (Chen et al., 2011; Grienberger et al., 2012; Sakata and Harris, 2009), that brief sound stimuli evoked prolonged depolarizing synaptic responses. These resemble spontaneous up states in terms of their peak amplitude values and durations (Figures S4A–S4C) but have a much faster rise time than that of spontaneous up states (Figures S4D–S4H; see also Zhang et al., 2011). We made recordings of both sound-evoked and spontaneous calcium transients (Figures 4A–4C). The results showed that spines that produced calcium transients in response to sound stimulation were also active during spontaneous up states (Figures 4B and 4C, spines S1 and S2), whereas silent spines were silent in both instances (Figures 4B and 4C, spine S3). Notably, we found a similar responsiveness of spine calcium responses during sound-evoked and spontaneous up states: the response rates of active spines were similar in both conditions, whereas silent spines were always silent (Figure 4D) (21 spines with high response rate, 56 spines with low response rate, and 12 silent

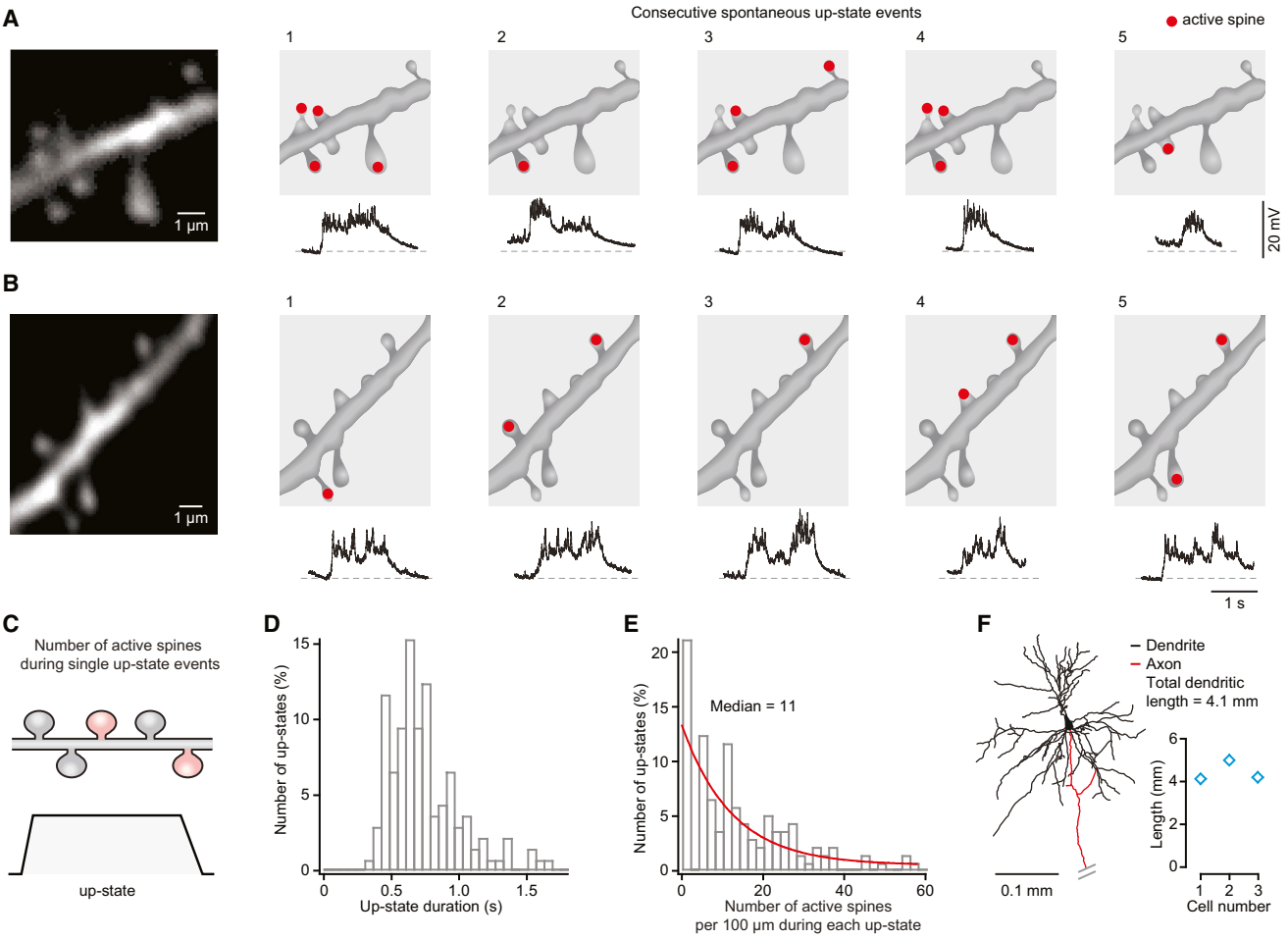

Figure 3. Number of Active Spines during Spontaneous Up States

(A and B) Left panels: two-photon images of dendritic segments, corresponding to the dendrites 1 and 2 in Figure 1F. Right panels: cartoons of these two dendritic segments with red dots indicating the spines that were activated during their corresponding up states.

(C) Schematic representation showing the number of active spines (marked in red) during a single up state.

(D) Distribution of the duration of up states (n = 150 up states from ten neurons).

(E) Distribution of the density of active spines (number of active spines per dendritic length) during single up states. The length of each imaged dendritic segment was normalized to 100 µm (n = 150 up states from ten neurons). The red line is an exponential fit to the data.

(F) Dendritic length of L2 cortical pyramidal neurons in the primary auditory cortex. Left: post hoc reconstruction of a biocytin-filled neuron (projection along the anteroposterior axis) with dendrites in black and the axons in red (the total dendritic length of this neuron = 4.1 mm). Note that for clarity, axon is truncated. Right: summary of dendritic lengths of three neurons.

See also Figure S3.

spines; n = 14 dendritic segments from ten neurons). As a control, we verified that action potentials (more than two action potentials) were able to produce calcium transients in all spines (Figure 4D, right-most panel). Another way of demonstrating the similar responsiveness of spine calcium responses is to compare the probability of exhibiting calcium responses in active spines over consecutive up states. As shown in Figure 4E, spines with a high response rate, defined by their responses to sensory stimulation, were always more frequently activated than spines with a low response rate across eight consecutive up states. A more detailed analysis of specific spines with a high response rate showed that during sound stimulation approximately two times more calcium transients occurred in the first 50 ms as compared with the activity during spontaneous up states. This may explain the faster rise time of sound-evoked up states (Figures S4F–S4H). Altogether, these results suggest that the same sets of synapses, reflecting action potential activity in the corresponding presynaptic neurons, are engaged with the same degree of reliability in both sound-evoked and spontaneous up-state activity. Notably, in line with these in vivo recordings, it has been found in thalamocortical slices that the same cortical neurons display similar spatiotemporal patterns in response to both spontaneous and thalamic stimulation-evoked up states (MacLean et al., 2005). Such a patterned activity may have important functional roles, because the recurrent calcium signals in the active spines during slow oscillations may

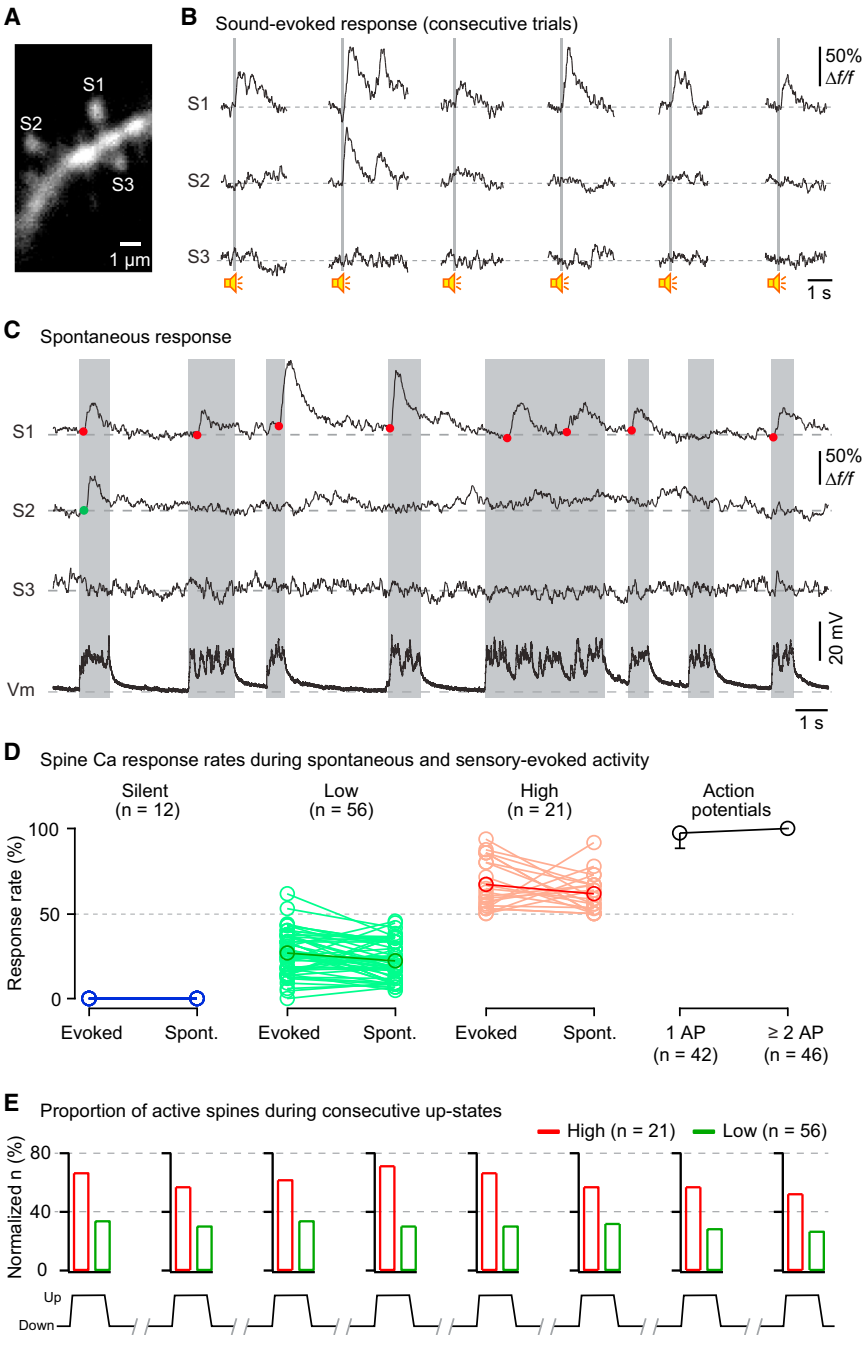

Figure 4. Similar Activity Profile of Spines during Sensory-Evoked and Spontaneous Up States

(A) Two-photon image of a dendritic segment (average of 6,250 frames) used for calcium imaging in (B).

(B) Calcium responses in spines (S1–S3) during sound stimulation (six consecutive trials). Gray bars indicate sound stimulation (broadband noise, 100 ms duration, 0 dB attenuation).

(C) Calcium responses in the same spines as shown in (B) (S1–S3) during spontaneous up states. Gray bars indicate duration of up states. Color dots indicate the onset of calcium responses. The neuron was held at the resting membrane potential.

(D) Comparison of the response rate of spine calcium responses during sound stimulation (Evoked), spontaneous up states (Spont.), and action potentials. The number of spines is indicated.

(E) Proportion of active spines, defined by the number of active spines during individual up states divided by the total number of spines in each class, over eight consecutive up states (n = 21 spines with high response rates marked in red and 56 spines with low response rates marked in green). See also Figure S4.

drive synaptic plasticity mechanisms related to memory consolidation of sensory information (Cavazzini et al., 2005; Diekelmann and Born, 2010; Nevian and Sakmann, 2006; Zucker, 1999).

Our results provide direct insights into the activity in dendritic spines during cortical up states in the absence of sensory stimulation. We observed subthreshold calcium transients in a large proportion of spines (90%) distributed throughout the dendritic field of L2 cortical neurons, whereas some spines (10%) were silent. A fraction of the active spines had a high response rate during spontaneous up states, whereas the other fraction had a low response rate. The large group of spines with a low response rate may mainly reflect inputs from other L2/3 cortical neurons, known to be sparsely active during up states (Chen et al., 2011; de Kock and Sakmann, 2008; Waters and Helmchen, 2006), whereas the spines with a high response rate may preferentially, but not exclusively, receive their inputs from deeper layers. In addition to the abundant spine calcium signaling observed during up states, we occasionally encountered spine calcium responses also during down states. This is an interesting finding suggesting the existence of synaptic inputs from subcortical neurons, because in the down state almost all cortical neurons are silent in terms of action potential activity (Steriade et al., 1993).

At the cortical circuit level, spontaneous and sensory-evoked up states are quite distinct in terms of the cellular origin and the propagation of the action potential activity (Sakata and Harris, 2009). For example, in the auditory cortex, sensory stimulation initiates the cortical network response by first activating layer 4, presumably through thalamocortical inputs, whereas spontaneous up-state-related activity is first observed in layer 5, most likely involving predominantly corticocortical connections. Here, we find that in L2 neurons the same sets of active synapses are engaged in both spontaneous up states and sensory

responses with similar response rates. Stereotypic motifs of local neuronal circuit activity, similar to those encountered during wakefulness, were reported to return repeatedly during slow wave activity (Luczak et al., 2007). Furthermore, there is strong evidence that ongoing cortical activity determines the brain's internal context containing attributes on memory, perception, and behavior (Kenet et al., 2003). Thus, a possible cellular mechanism underlying memory consolidation in cortical neurons may involve the repeated activation of those spines that are activated during sensory stimulation.

We showed that subthreshold single spine calcium signals are strictly NMDAR dependent, during both spontaneous (Figures 2A and 2B) and sound-stimulation-evoked activity (Chen et al., 2011). In view of the essential role of spine calcium signaling (Nevian and Sakmann, 2006; Sabatini et al., 2001) and NMDAR activation (Lee et al., 2009; Matsuzaki et al., 2004) for experience-dependent synaptic plasticity in cortical neurons, these patterned spine calcium signals during spontaneous up states are well suited as reinforcement signals underlying the cellular transformations associated with sleep-related memory consolidation (Diekelmann and Born, 2010; Marshall et al., 2006; Stickgold, 2005).

EXPERIMENTAL PROCEDURES

All experimental procedures were performed in accordance with institutional animal welfare guidelines and were approved by the state government of Bavaria, Germany. C57BL/6 mice (30–50 postnatal days) were prepared under isoflurane anesthesia for two-photon calcium imaging and whole-cell patch-clamp recordings as described previously (Chen et al., 2011, 2012; Jia et al., 2010). The patch pipette was used for loading the fluorescent calcium indicator, Oregon Green BAPTA-1 Hexapotassium (OGB-1, 120 μM), into layer 2 cortical pyramidal neurons in the primary auditory cortex. Dendritic spines were imaged in vivo with the LOTOS procedure using a custom-built acousto-optic-deflector (AOD)-based two-photon microscope as recently described (Chen et al., 2011, 2012). In brief, the LOTOS-based procedure allowed acquirement of images at a frame rate of 1,000 frames/s^{-1} with 50 ns pixel dwell time and low excitation intensity. Auditory responses were evoked by 100 ms sound stimuli (10 ms rise/fall time) via an ES1 (Tucker-David Technologies) free-field speaker. For data analysis, the original image data that were acquired at a sampling rate of 1,000 frames/s^{-1} were downsampled 10- to 12-fold (to 80–100 images s^{-1}). Calcium signals were expressed as relative fluorescence changes ($\Delta f/f$) corresponding to the mean fluorescence from all pixels within specified regions of interest (ROIs). The traces were further smoothed with an exponentially averaging IIR filter (time constant: 80 ms) as described previously (Chen et al., 2011; Jia et al., 2010). In some recordings, calcium signals were observed in both dendrites and spines during action potential firing, which was not due to signals from the dendritic shafts scattering into the spine regions. This is because our procedure allows for significant separation of these signals (Figure S5). Statistical analyses were performed using paired or unpaired Student's t test as appropriate. A p value <0.05 was considered as significant. Six neurons analyzed in this work have been already reported for different questions previously (Chen et al., 2011): two neurons were in the conditions of control recordings, and four were in the presence of MK-801. More detailed description of experimental procedures is available in the Extended Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.05.042.

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